



FIG. 1

sequence_AA_2012.5_variable domains.tkv

>2012.5VL_MOUSE
QAVVTQESALTTSPGETVLTCSRSTGAVTTSNYANWVQEKPDHLFTGLIGNNRPPGYPARFSGSLIGDKAALTIAGTQTED (SEQ ID NO: 1)
EATYFCALWYSNHWVFGGTRLTVLG

CDR1 - RSSTGAVTTSNYAN (SEQ ID NO: 2)
CDR2 - GNNRPP (SEQ ID NO: 3)
CDR3 - ALWYSNHWV (SEQ ID NO: 4)

>2012.5VH_MOUSE
QVKLQESGPGLVQPSQSLSITCTVSGFSLTDYGVHWRQSPGKGLEWLGVWSSGGTAYTAAFISRLNIY (SEQ ID NO: 5)
KNSKNQVFFEMNSLQANDTAMYCCARRGSPYNYFDVWGGTTTVTVSS

CDR1 - DYGVH (SEQ ID NO: 6)
CDR2 - VIWSSGGTAYTAAFIS (SEQ ID NO: 7)
CDR3 - RGSYPYNYFDV (SEQ ID NO: 8)

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2d12.5 VH native hybridoma = SEQ ID NO: 15
 2d12.5 VH native cloned = SEQ ID NO: 16
 2d12.5 VH N87D_cloned = SEQ ID NO: 17

2d12.5 VH N87D_G53C_cloned = SEQ ID NO: 18
 2d12.5 VH N87D_G54C_cloned = SEQ ID NO: 19
 2d12.5 VH N87D_G55C_cloned = SEQ ID NO: 20

FIG. 3A

2D12.5 VH variable genes

2d12.5 VH native hybridoma	110.....20.....30.....40.....50	
2d12.5 VH native cloned	1	GTGAAGCTGCAGGAGTCAGGACCTGGCCTAGTGCAGCCCTCACAGGCCT	50
2d12.5 VH N87D_cloned	1T.....	50
2d12.5 VH N87D_G53C_cloned	1T.....	50
2d12.5 VH N87D_G54C_cloned	1T.G.....	50
2d12.5 VH N87D_G55C_cloned	1T.....	50
2d12.5 VH native hybridoma	5160.....70.....80.....90.....100	
2d12.5 VH native cloned	51	GTCCATCACCTGCACGGTCTCTGGTTTCTCATTAAGTACTATGGTGATC	100
2d12.5 VH N87D_cloned	51	100
2d12.5 VH N87D_G53C_cloned	51	100
2d12.5 VH N87D_G54C_cloned	51	100
2d12.5 VH N87D_G55C_cloned	51	100
2d12.5 VH native hybridoma	101110.....120.....130.....140.....150	
2d12.5 VH native cloned	101	ACTGGGTTTCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGAGTGATA	150
2d12.5 VH N87D_cloned	101	150
2d12.5 VH N87D_G53C_cloned	101	150
2d12.5 VH N87D_G54C_cloned	101	150
2d12.5 VH N87D_G55C_cloned	101	150
2d12.5 VH native hybridoma	151160.....170.....180.....190.....200	
2d12.5 VH native cloned	151	TGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTTCATATCCAGACTGAA	200
2d12.5 VH N87D_cloned	151	200
2d12.5 VH N87D_G53C_cloned	151T.....	200
2d12.5 VH N87D_G54C_cloned	151T.T.....	200
2d12.5 VH N87D_G55C_cloned	151T.....	200
2d12.5 VH native hybridoma	201210.....220.....230.....240.....250	
2d12.5 VH native cloned	201	CATCTACAAGGACAAATCCAAAGACCAAGTTTCTTTGAAATGAACAGTC	250
2d12.5 VH N87D_cloned	201	250
2d12.5 VH N87D_G53C_cloned	201	250
2d12.5 VH N87D_G54C_cloned	201	250
2d12.5 VH N87D_G55C_cloned	201	250
2d12.5 VH native hybridoma	251260.....270.....280.....290.....300	
2d12.5 VH native cloned	251	TGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGTAGCTAC	300
2d12.5 VH N87D_cloned	251G.....	300
2d12.5 VH N87D_G53C_cloned	251G.....	300
2d12.5 VH N87D_G54C_cloned	251G.....	300
2d12.5 VH N87D_G55C_cloned	251G.....	300
2d12.5 VH native hybridoma	301310.....320.....330.....340.....350	
2d12.5 VH native cloned	301	CCTTACAACTACTTCGATGTCTGGGGCCAAAGGACCACAGTACCGTCTC	350
2d12.5 VH N87D_cloned	301G.....	350
2d12.5 VH N87D_G53C_cloned	301G.....	350
2d12.5 VH N87D_G54C_cloned	301G.....	350
2d12.5 VH N87D_G55C_cloned	301G.....	350

FIG. 3B

2D12.5 VH variable genes

2d12.5 VH native hybridoma	351	354
2d12.5 VH native cloned	351	CTCA	354
2d12.5 VH N87D_cloned	351	.G..	354
2d12.5 VH N87D_G53C_cloned	351	.G..	354
2d12.5 VH_N87D_G54C_cloned	351	.G..	354
2d12.5 VH N87D_G55C_cloned	351	.G..	354

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FIG. 4

Translation of 2d12.5 VL genes

(SEQ ID NO: 21)	2d12.5 VL native hybridoma	1	10	20	30	40	50
(SEQ ID NO: 22)	2d12.5 VL native cloned	1	AVVTQESALTTSFGETVTLTCRSSTGAVTTSNYANWVQKPDHLFTGLIG					
(SEQ ID NO: 23)	2d12.5 VL N53C_cloned	1	10	20	30	40	50
			60	70	80	90	100
	2d12.5 VL native hybridoma	51	GNNRPPGVFPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWVFG					
	2d12.5 VL native cloned	51	60	70	80	90	100
	2d12.5 VL N53C_cloned	51	.C.....	60	70	80	90	100
			60	70	80	90	100
	2d12.5 VL native hybridoma	101	GGTRLTVLG					
	2d12.5 VL native cloned	101K....S					
	2d12.5 VL N53C_cloned	101K....S					

FIG. 5

2012.5 VL variable genes

[illegible]

FIG. 6

Translation of Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

(SEQ ID NO: 27) 2dVL-TTCL native_cloned	1	10	20	30	40	50
(SEQ ID NO: 28) 2dVL-TTCL N53C_cloned	1	RS	AVVTQESALTTSPGETVTLTCRSSSTGAVTTSNYANVWQKPDHLFTGL	50		
(SEQ ID NO: 29) 2d12.5 VL native hybridoma	1					50
(SEQ ID NO: 30) TTCL template for gene assembly	1					48
						1
2dVL-TTCL native_cloned	51	60	70	80	90	100
2dVL-TTCL N53C_cloned	51	IGGNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWV	100			
2d12.5 VL native hybridoma	49					100
TTCL template for gene assembly	1					98
						1
2dVL-TTCL native_cloned	101	110	120	130	140	150
2dVL-TTCL N53C_cloned	101	FGGGTKLTVLSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV				
2d12.5 VL native hybridoma	99					
TTCL template for gene assembly	1					
2dVL-TTCL native_cloned	151	160	170	180	190	200
2dVL-TTCL N53C_cloned	151	QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV	200			
2d12.5 VL native hybridoma						200
TTCL template for gene assembly	40					89
2dVL-TTCL native_cloned	201	210	220			
2dVL-TTCL N53C_cloned	201	THQGLSLPVTKSFNRGEC*F*	221			
2d12.5 VL native hybridoma						221
TTCL template for gene assembly	90					107

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2dVL-TTCL native_cloned = SEQ ID NO: 31

2dVL-TTCL N53C_cloned = SEQ ID NO: 32

2d12.5 VL native hybridoma = SEQ ID NO: 33

TTCL template for gene assem = SEQ ID NO: 34

FIG. 7A

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

		10	20	30	40	50
2dVL-TTCL native_cloned	1	AGATCTGCTGTTGTGACTCAGGAATCTGCACTCACCACATCACCCTGGTGA	50			
2dVL-TTCL N53C_cloned	1	50			
2d12.5 VL native hybridoma	1	44			
TTCL template for gene assem	1				
		60	70	80	90	100
2dVL-TTCL native_cloned	51	AACAGTCACACTCACTTGTGCTCAAGTACTGGGGCTGTTACGACTAGTA	100			
2dVL-TTCL N53C_cloned	51	100			
2d12.5 VL native hybridoma	45	94			
TTCL template for gene assem	1				
		110	120	130	140	150
2dVL-TTCL native_cloned	101	ACTATGCCAACTGGGTCCAAGAGAAACCAGATCATTATTCAGTGGTCTA	150			
2dVL-TTCL N53C_cloned	101	150			
2d12.5 VL native hybridoma	95	144			
TTCL template for gene assem	1				
		160	170	180	190	200
2dVL-TTCL native_cloned	151	ATAGGTGGTAATAATAACCGACCTCCAGGTGTTCTCCGACGATTCTCAGG	200			
2dVL-TTCL N53C_cloned	151TG.....	200			
2d12.5 VL native hybridoma	145	194			
TTCL template for gene assem	1				
		210	220	230	240	250
2dVL-TTCL native_cloned	201	CTCCCTGATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTG	250			
2dVL-TTCL N53C_cloned	201	250			
2d12.5 VL native hybridoma	195	244			
TTCL template for gene assem	1				
		260	270	280	290	300
2dVL-TTCL native_cloned	251	AGGATGAGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTGGGTG	300			
2dVL-TTCL N53C_cloned	251	300			
2d12.5 VL native hybridoma	245	294			
TTCL template for gene assem	1				
		310	320	330	340	350
2dVL-TTCL native_cloned	301	TTCGGTGGGGGAACCAAACTGACTGTCTTAAGCCGAACTGTGGCTGCACC	350			
2dVL-TTCL N53C_cloned	301	350			
2d12.5 VL native hybridoma	295A.....G.....G.....				
TTCL template for gene assem	1				
		360	370	380	390	400
2dVL-TTCL native_cloned	351	ATCTGTCTTCATCTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTG	400			
2dVL-TTCL N53C_cloned	351	400			
2d12.5 VL native hybridoma					
TTCL template for gene assem	18	61			
		410	420	430	440	45
2dVL-TTCL native_cloned	401	CCTCTGTTGTGTGCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTA				
2dVL-TTCL N53C_cloned	401				
2d12.5 VL native hybridoma					
TTCL template for gene assem	68				

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FIG. 7B

Mouse 2d12.5 VL - Human TetTox CL kappa (light chain gene,

2dVL-TTCL native_cloned	451	460	470	480	490	500
2dVL-TTCL N53C_cloned	451
2d12.5 VL native hybridoma	
TTCL template for gene assem	118	167
2dVL-TTCL native_cloned	501	510	520	530	540	550
2dVL-TTCL N53C_cloned	501
2d12.5 VL native hybridoma	
TTCL template for gene assem	168	217
2dVL-TTCL native_cloned	551	560	570	580	590	600
2dVL-TTCL N53C_cloned	551
2d12.5 VL native hybridoma	
TTCL template for gene assem	218	267
2dVL-TTCL native_cloned	601	610	620	630	640	650
2dVL-TTCL N53C_cloned	601
2d12.5 VL native hybridoma	
TTCL template for gene assem	268	317
2dVL-TTCL native_cloned	651	660				
2dVL-TTCL N53C_cloned	651
2d12.5 VL native hybridoma	
TTCL template for gene assem	318	322

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FIG. 8

Translation of Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH native cloned	1	RSVKIQBSGPGLVQPSQSLSTCTVSGFSLIDYGVHWVRQSPKGLEWLG	50	(SEQ ID NO: 35)
2dVH-TTCH_N87D_cloned	1	50	(SEQ ID NO: 36)
2dVH-TTCH_N87D_G53C_cloned	1	50	(SEQ ID NO: 37)
2dVH-TTCH_N87D_G54C_cloned	1	50	(SEQ ID NO: 38)
2dVH-TTCH_N87D_G55C_cloned	1	50	(SEQ ID NO: 39)
2dVH-TTCH expected sequence	1	50	(SEQ ID NO: 40)
2d12.5 VH native hybridoma	1	48	(SEQ ID NO: 41)
2dVH-TTCH native cloned	51	100	
2dVH-TTCH_N87D_cloned	51	VIWGGGTAYTAAFISRLNIYKDKSNQVFFEMNSLQANDTAMTYCARRG	100	
2dVH-TTCH_N87D_G53C_cloned	51	100	
2dVH-TTCH_N87D_G54C_cloned	51	100	
2dVH-TTCH_N87D_G55C_cloned	51	100	
2dVH-TTCH expected sequence	51	100	
2d12.5 VH native hybridoma	49	98	
2dVH-TTCH native cloned	101	SYFYNFVWGQGTIVTVAASATKGFVFLA:SSKSTSGGTAALGCLVK	150	
2dVH-TTCH_N87D_cloned	101	150	
2dVH-TTCH_N87D_G53C_cloned	101	150	
2dVH-TTCH_N87D_G54C_cloned	101	150	
2dVH-TTCH_N87D_G55C_cloned	101	150	
2dVH-TTCH expected sequence	101	150	
2d12.5 VH native hybridoma	99S	118	
2dVH-TTCH native cloned	151	DYFPEPVTVMNSGALTSGVHTFPAVLQSSGLYSLSGVTVFSSSLGTQT	200	
2dVH-TTCH_N87D_cloned	151	200	
2dVH-TTCH_N87D_G53C_cloned	151	200	
2dVH-TTCH_N87D_G54C_cloned	151	200	
2dVH-TTCH_N87D_G55C_cloned	151	200	
2dVH-TTCH expected sequence	151	200	
2d12.5 VH native hybridoma		200	
2dVH-TTCH native cloned	201	YICNVHKKPSNTKVDKKAEPKSCDKSR	227	
2dVH-TTCH_N87D_cloned	201	227	
2dVH-TTCH_N87D_G53C_cloned	201	227	
2dVH-TTCH_N87D_G54C_cloned	201	227	
2dVH-TTCH_N87D_G55C_cloned	201	227	
2dVH-TTCH expected sequence	201	227	
2d12.5 VH native hybridoma		227	

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2dVH-TTCH_native cloned = SEQ ID NO: 42
 2dVH-TTCH_N87D_cloned = SEQ ID NO: 43
 2dVH-TTCH_N87D_G53C_cloned = SEQ ID NO: 44
 2dVH-TTCH_N87D_G54C_cloned = SEQ ID NO: 45
 2dVH-TTCH_N87D_G55C_cloned = SEQ ID NO: 46

2dVH-TTCH expected sequence = SEQ ID NO: 47

FIG. 9A 2d12.5 VH native hybridoma = SEQ ID NO: 48

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

		10	20	30	40	50
2dVH-TTCH_native cloned	1	AGATCTGTGAAGCTGCAGGAGTCTGGACCTGGCCTAGTGCAGCCCTCACA	50			
2dVH-TTCH_N87D_cloned	1	50			
2dVH-TTCH_N87D_G53C_cloned	1	50			
2dVH-TTCH_N87D_G54C_cloned	1G.....	50			
2dVH-TTCH_N87D_G55C_cloned	1	50			
2dVH-TTCH expected sequence	1	50			
2d12.5 VH native hybridoma	1A.....	44			
		60	70	80	90	100
2dVH-TTCH_native cloned	51	GAGCCTGTCCATCACCTGCACGGTCTCTGGTTCTCATTAACTGACTATG	100			
2dVH-TTCH_N87D_cloned	51	100			
2dVH-TTCH_N87D_G53C_cloned	51	100			
2dVH-TTCH_N87D_G54C_cloned	51	100			
2dVH-TTCH_N87D_G55C_cloned	51	100			
2dVH-TTCH expected sequence	51	100			
2d12.5 VH native hybridoma	45	94			
		110	120	130	140	150
2dVH-TTCH_native cloned	101	GTGTACACTGGGTTCCGCCAGTCTCCAGGAAAGGGTCTGGAATGGCTGGGA	150			
2dVH-TTCH_N87D_cloned	101	150			
2dVH-TTCH_N87D_G53C_cloned	101	150			
2dVH-TTCH_N87D_G54C_cloned	101	150			
2dVH-TTCH_N87D_G55C_cloned	101	150			
2dVH-TTCH expected sequence	101	150			
2d12.5 VH native hybridoma	95	144			
		160	170	180	190	200
2dVH-TTCH_native cloned	151	GTGTATATGGAGTGGTGGAGGCACGGCCTATACTGCGGCGTTCATATCCAG	200			
2dVH-TTCH_N87D_cloned	151	200			
2dVH-TTCH_N87D_G53C_cloned	151T.....	200			
2dVH-TTCH_N87D_G54C_cloned	151T.T.....	200			
2dVH-TTCH_N87D_G55C_cloned	151T.....	200			
2dVH-TTCH expected sequence	151	200			
2d12.5 VH native hybridoma	145	194			
		210	220	230	240	250
2dVH-TTCH_native cloned	201	ACTGAACATCTACAAGGACAATTCCAAGAACCAAGTTTCTTTGAAATGA	250			
2dVH-TTCH_N87D_cloned	201	250			
2dVH-TTCH_N87D_G53C_cloned	201	250			
2dVH-TTCH_N87D_G54C_cloned	201	250			
2dVH-TTCH_N87D_G55C_cloned	201	250			
2dVH-TTCH expected sequence	201	250			
2d12.5 VH native hybridoma	195	244			
		260	270	280	290	300
2dVH-TTCH_native cloned	251	ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT	300			
2dVH-TTCH_N87D_cloned	251G.....	300			
2dVH-TTCH_N87D_G53C_cloned	251G.....	300			
2dVH-TTCH_N87D_G54C_cloned	251G.....	300			
2dVH-TTCH_N87D_G55C_cloned	251G.....	300			
2dVH-TTCH expected sequence	251	300			
2d12.5 VH native hybridoma	245	294			
		310	320	330	340	350
					

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FIG. 9B

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH_native cloned	301	AGCTACCCTTACAACCTACTTTCGATGTCTGGGGCCAAAGGGACCAACGGTCAC	350
2dVH-TTCH_N87D_cloned	301	350
2dVH-TTCH_N87D_G53C_cloned	301	350
2dVH-TTCH_N87D_G54C_cloned	301	350
2dVH-TTCH_N87D_G55C_cloned	301	350
2dVH-TTCH expected sequence	301	350
2d12.5 VH native hybridoma	295A.....	344

		360	370	380	390	400	
2dVH-TTCH_native cloned	351	CGTCTCCGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCT	406				
2dVH-TTCH_N87D_cloned	351	406				
2dVH-TTCH_N87D_G53C_cloned	351	406				
2dVH-TTCH_N87D_G54C_cloned	351	406				
2dVH-TTCH_N87D_G55C_cloned	351	406				
2dVH-TTCH expected sequence	351	400				
2d12.5 VH native hybridoma	345T..	354				

		410	420	430	440	450	
2dVH-TTCH_native cloned	401	CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG	450				
2dVH-TTCH_N87D_cloned	401	450				
2dVH-TTCH_N87D_G53C_cloned	401	450				
2dVH-TTCH_N87D_G54C_cloned	401	450				
2dVH-TTCH_N87D_G55C_cloned	401	450				
2dVH-TTCH expected sequence	401	450				
2d12.5 VH native hybridoma							

		460	470	480	490	500	
2dVH-TTCH_native cloned	451	GACTACTTCCCCGAACCGGTGACGGTGTCTTGGAACTCAGGCGCCCTGAC	500				
2dVH-TTCH_N87D_cloned	451	500				
2dVH-TTCH_N87D_G53C_cloned	451	500				
2dVH-TTCH_N87D_G54C_cloned	451	500				
2dVH-TTCH_N87D_G55C_cloned	451	500				
2dVH-TTCH expected sequence	451G.....	500				
2d12.5 VH native hybridoma							

		510	520	530	540	550	
2dVH-TTCH_native cloned	501	CAGCGGCGTGACACCTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACT	550				
2dVH-TTCH_N87D_cloned	501	550				
2dVH-TTCH_N87D_G53C_cloned	501	550				
2dVH-TTCH_N87D_G54C_cloned	501	550				
2dVH-TTCH_N87D_G55C_cloned	501	550				
2dVH-TTCH expected sequence	501	550				
2d12.5 VH native hybridoma							

		560	570	580	590	600	
2dVH-TTCH_native cloned	551	CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC	600				
2dVH-TTCH_N87D_cloned	551	600				
2dVH-TTCH_N87D_G53C_cloned	551	600				
2dVH-TTCH_N87D_G54C_cloned	551	600				
2dVH-TTCH_N87D_G55C_cloned	551	600				
2dVH-TTCH expected sequence	551	600				
2d12.5 VH native hybridoma							

		610	620	630	640	650	
2dVH-TTCH_native cloned	601	TACATCTGCAACGTGAATCACAAGCCCAAGCAACCAAGGTGGACAAGAA	650				
2dVH-TTCH_N87D_cloned	601	650				

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

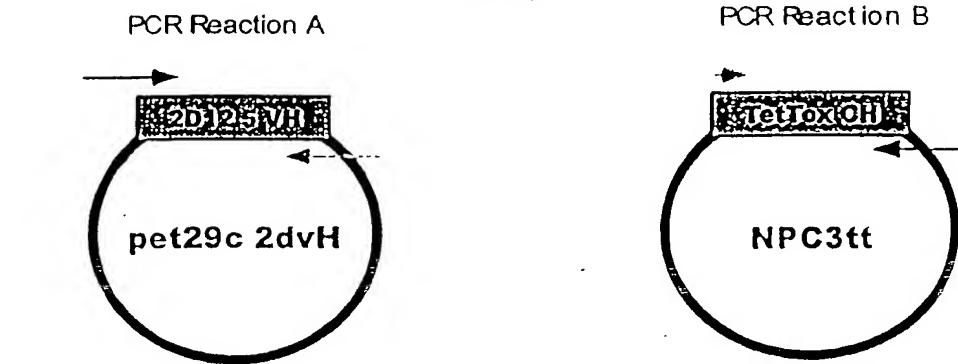
[illegible]

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FIG. 10A

Strategy for Assembly of Chimeric 2D12.5 Heavy Chain

Step 1



PCR Products

A

B

PCR cycle - 2DTTover

1 - 94° C 10 min

2 - 94° C 1 min

3 - 62° C 1 min

4 - 72° C 2 min

5 - 25x to step 2

6 - 72° C 5 min

7 - 4° C 18 hr

8 - END

Taq added before step 2

Example reaction

d2H2O 63 uL

10x buffer 10 uL

25mM MgCl₂ 8 uL

2mM dNTP 10 uL

Template 1 (1 ng/ uL) 1 uL

Top Primer 25 pmol/ uL 4 uL

Bottom Primer 25 pmol/ uL 4 uL

Taq Polymerase 0.5 uL

Primers

→ VH2D5' BglII (SEQ ID NO: 49)
GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← VH2DTTMI (SEQ ID NO: 51)
GCCCTTGGTGGAGGCTGCCGAGACGGTGACC-
GTGG

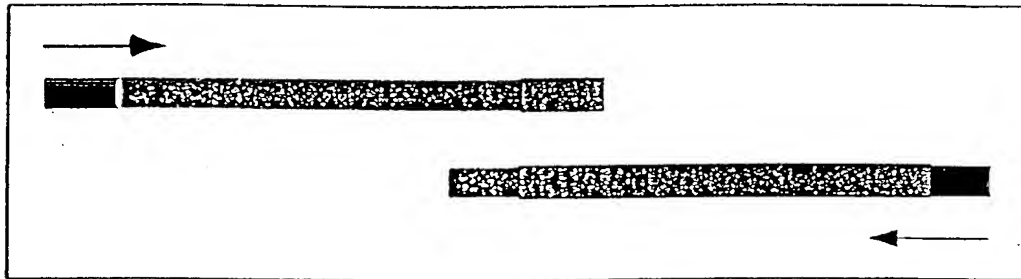
→ CHTT2DMI (SEQ ID NO: 50)
CCACGGTCACCGTCTCCGAGCCTCCAC-
CAAGGGC

← TTCH3' Xba (SEQ ID NO: 52)
CGATCTAGATTTGTCACAAGATTTGGGCTCTGC

FIG. 10B

Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 2

PCR Reaction



PCR cycle - 2DTTVent

- 1 - 95°C 10 min
 - 2 - 94°C 1 min
 - 3 - 60°C 1 min
 - 4 - 75°C 2 min
 - 5 - 4x to step 2
 - 6 - 94°C 1 min
 - 7 - 63°C 1 min
 - 8 - 75°C 2 min
 - 9 - 25x to step 6
 - 10 - 72°C 5 min
 - 11 - 4°C 18 hr
 - 12 - END
- Vent added before step 2
Primers added before step 6

Primers

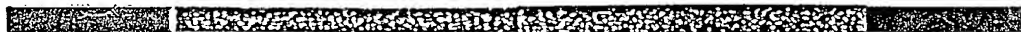
→ VH2D5' BglII (SEQ ID NO: 49)
 GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

 ← TTCH3' Xba (SEQ ID NO: 52)
 CGATCTAGATTGTCAAGATTGGGCTCTGC

Example reaction

d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template 1(1ng/ uL)	1uL
Template 2(1ng/ uL)	1uL
Top Primer 25pmol/ uL	4uL
Bottom Primer 25pmol/ uL	4uL
Vent Polymerase	0.5uL

PCR Assembly Product



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FIG. 10C

Strategy for Assembly of Chimeric 2D12.5 Heavy Chain Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

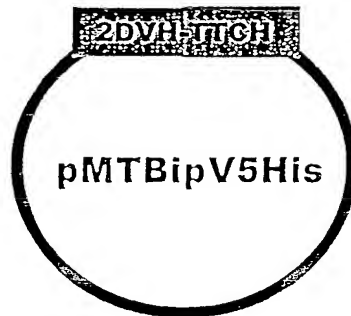
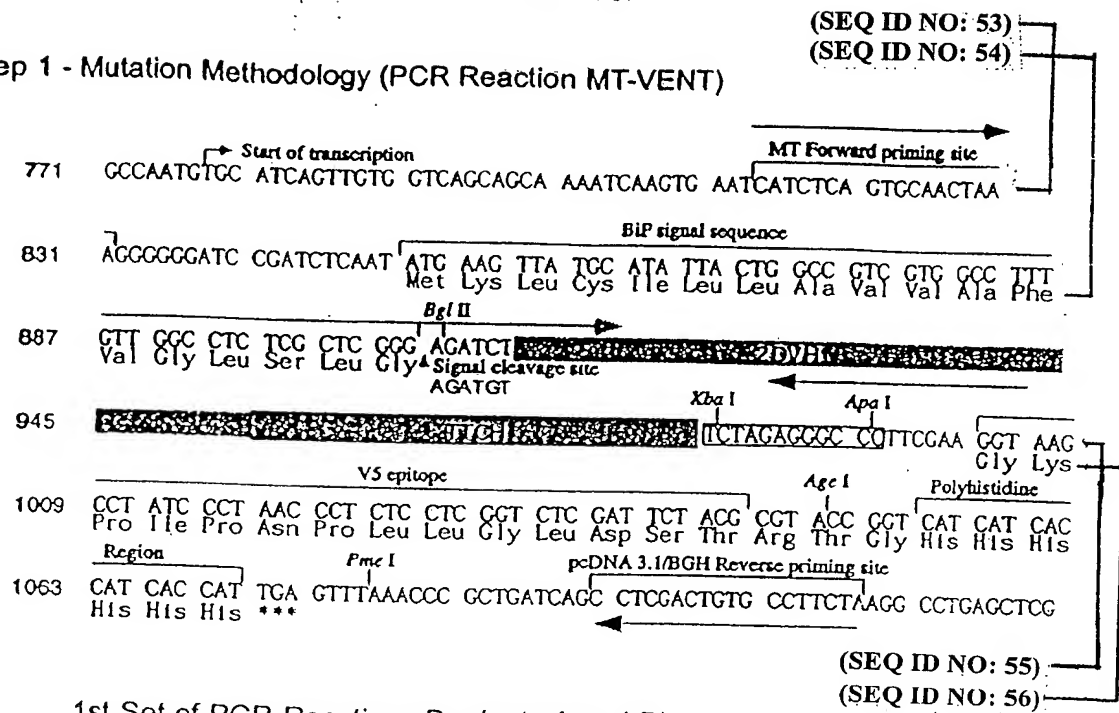


FIG. 10D

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



1st Set of PCR Reactions Product A and B)

Rxn A

Mutation

CATCTCAGTGCAACTAAA (SEQ ID NO: 57) MTforward

CATGGCTGTGTGTCATCAGCTTGCAGACTGTTC (SEQ ID NO: 58) 2dvhN87D_pMTBip

OR

CGTGCTCCACAACCTCCATATCAC (SEQ ID NO: 59) G53C noncoding 2dG53c_pMT

OR

CCGTGCCACAACCACTCCATATC (SEQ ID NO: 60) G54C noncoding 2dG54c_pMT

OR

CCGTGCATCCACCACTCCATATC (SEQ ID NO: 61) G55C noncoding 2dG55c_pMT

Killed Bgl II

Rxn B

GCTCGGGAGATGTGTGAAGCTG (SEQ ID NO: 62) 2dvhKBgIII_pMTBip

TAGAAGGCACAGTCGAGG (SEQ ID NO: 63) BGHreverse

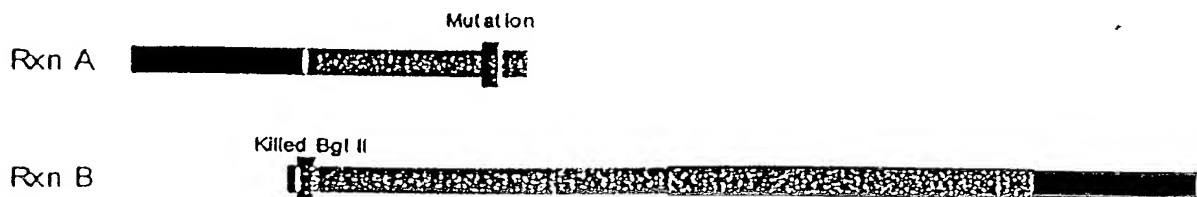
FIG. 10E

Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

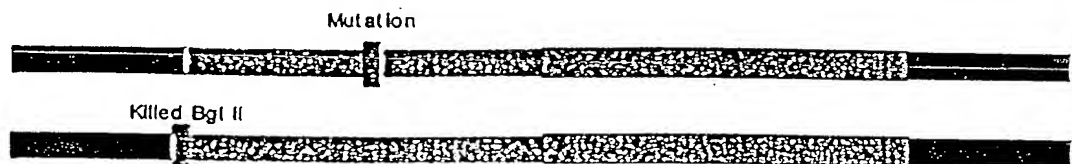
2nd PCR Reaction (Mix Products of reaction A and B)

1) Extend

2) Amplify with outer primers (MTforward and BGHreverse)



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

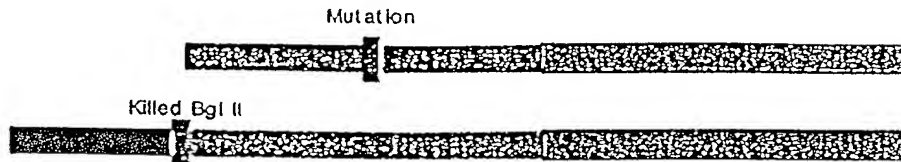


FIG. 10F

Step 1 - PCR Reaction MT-VENT

PCR cycle - MT-VENT

1 - 95°C 10 min
 2 - 94°C 1 min
 3 - 50°C 1 min
 4 - 75°C 2 min
 5 - 24x to step 2
 6 - 75°C 5 min
 7 - 4°C 18 hr
 8 - END
 VENT added before step 2
 Primers added before step 1

Example reaction

d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Vent Polymerase	0.5uL

Step 2 - PCR Reaction VHMUTTAQ

PCR cycle - VHMUTTAQ

1 - 95°C 10 min
 2 - 94°C 1 min
 3 - 68°C 1 min
 4 - 72°C 2 min
 5 - 4x to step 2
 6 - 94°C 1 min
 7 - 50°C 1 min
 8 - 72°C 2 min
 9 - 24x to step 6
 10 - 72°C 5 min
 11 - 4°C 18 hr
 12 - END
 Taq added before step 2
 Primers added before step 6

Example reaction

d2H2O	61ul
10x buffer	10uL
25mM MgCl2	8uL
2mM dNTP	10uL
Template 1 (1ng/uL)	1uL
Template 2 (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Taq Polymerase	0.5uL

FIG. 11A

Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 1

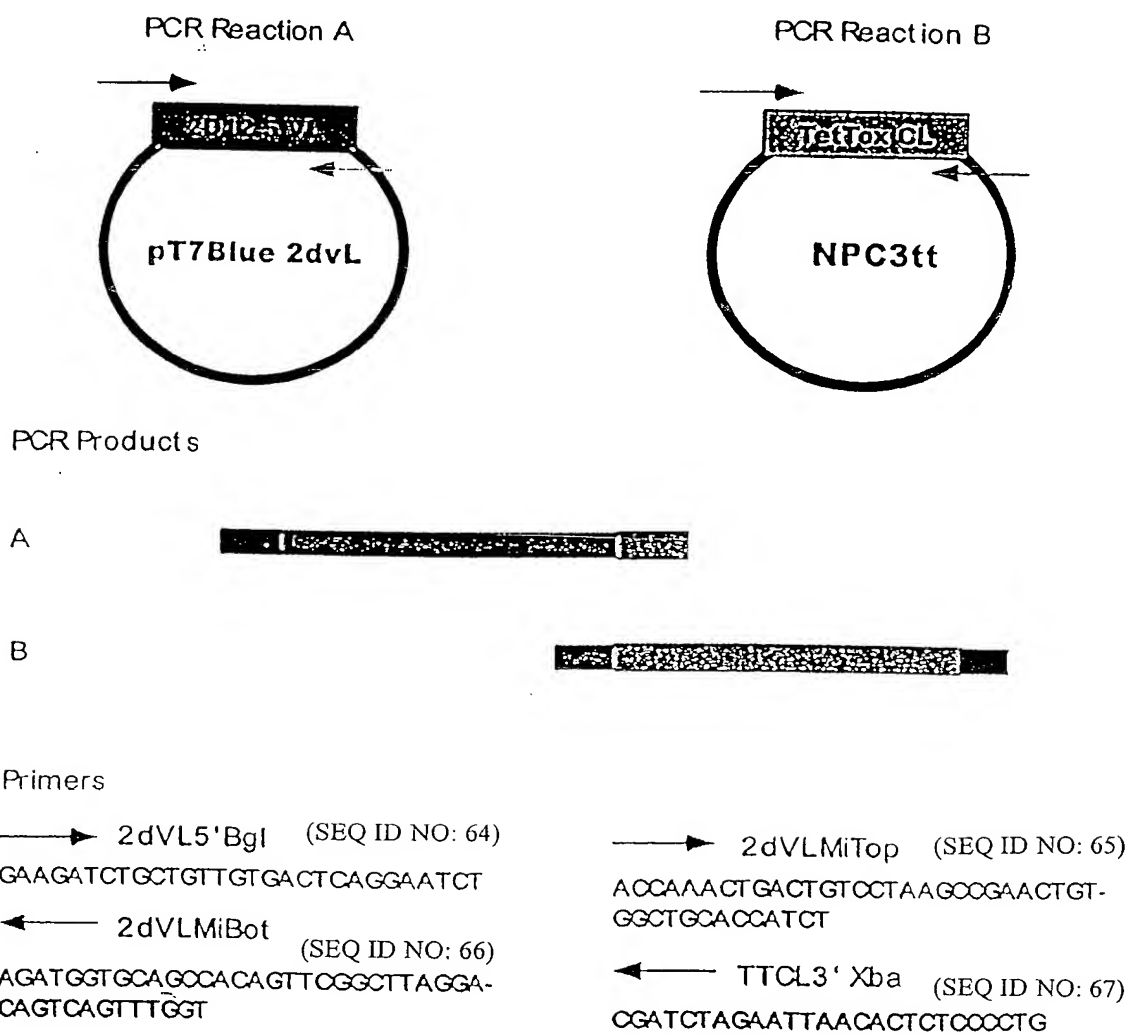
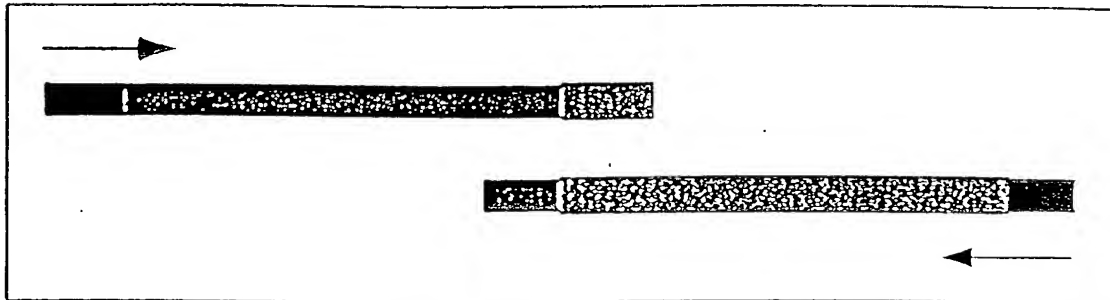


FIG. 11B

Strategy for Assembly of Chimeric 2D12.5
Light Chain
Step 2
PCR Reaction



Primers

→ 2dVL5'Bgl (SEQ ID NO: 64)

GAAGATCTGCTGTTGTGACTCAGGAATCT

← TTCL3' Xba (SEQ ID NO: 67)

CGATCTAGAATTAACACTCTCCCTG

PCR Assembly Product

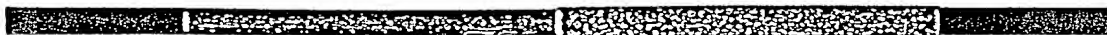


FIG. 11C

Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

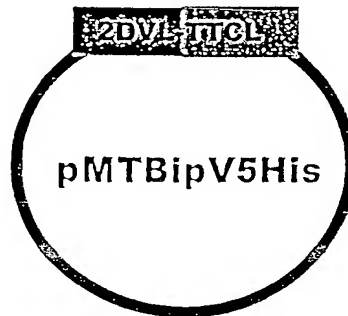
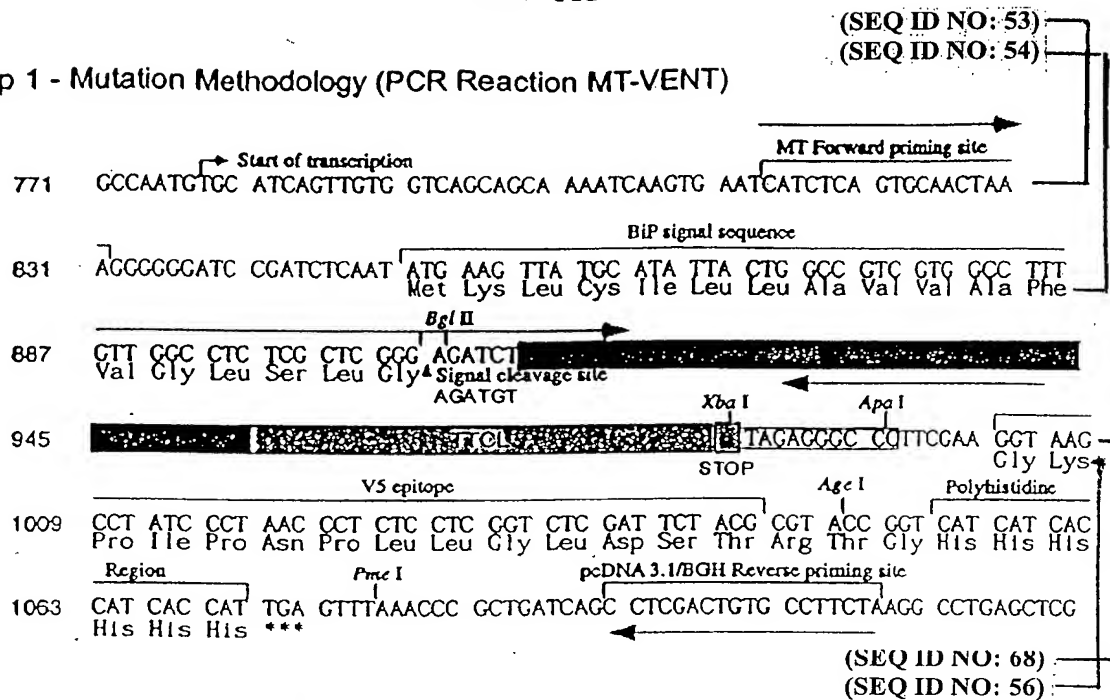


FIG. 11D

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



1st Set of PCR Reactions Producta A and B)

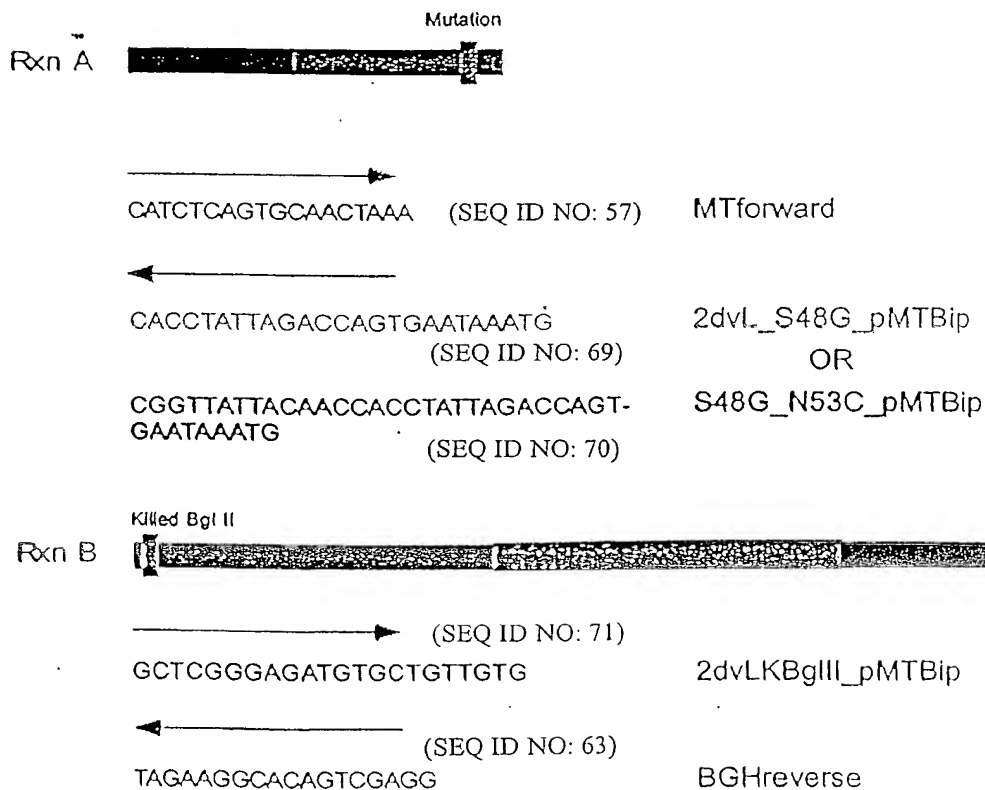


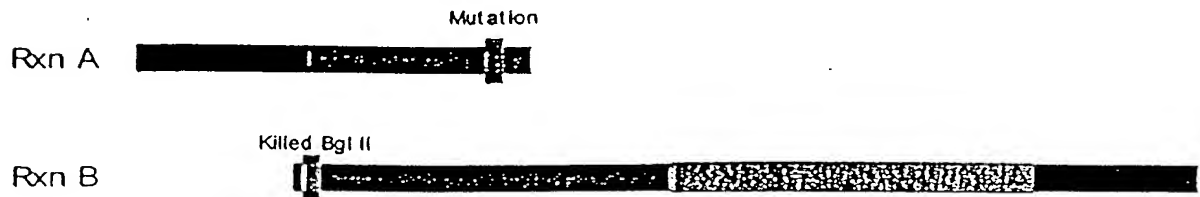
FIG. 11E

Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

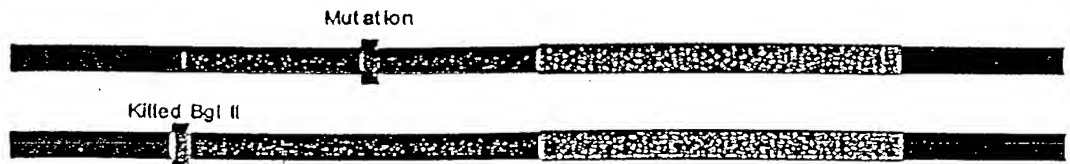
2nd PCR Reaction Mix Products of reaction B)

1) Extend

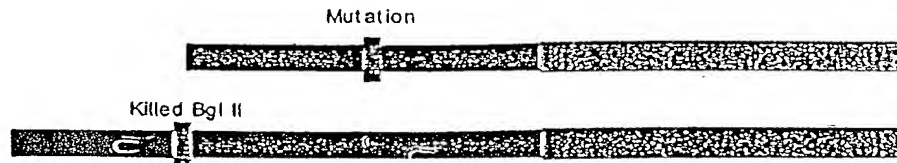
2) Amplify with outer primers (MTforward and BGHreverse)



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1



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FIG. 11F

Strategy for Assembly of Chimeric 2D12.5 Light Chain Step 4

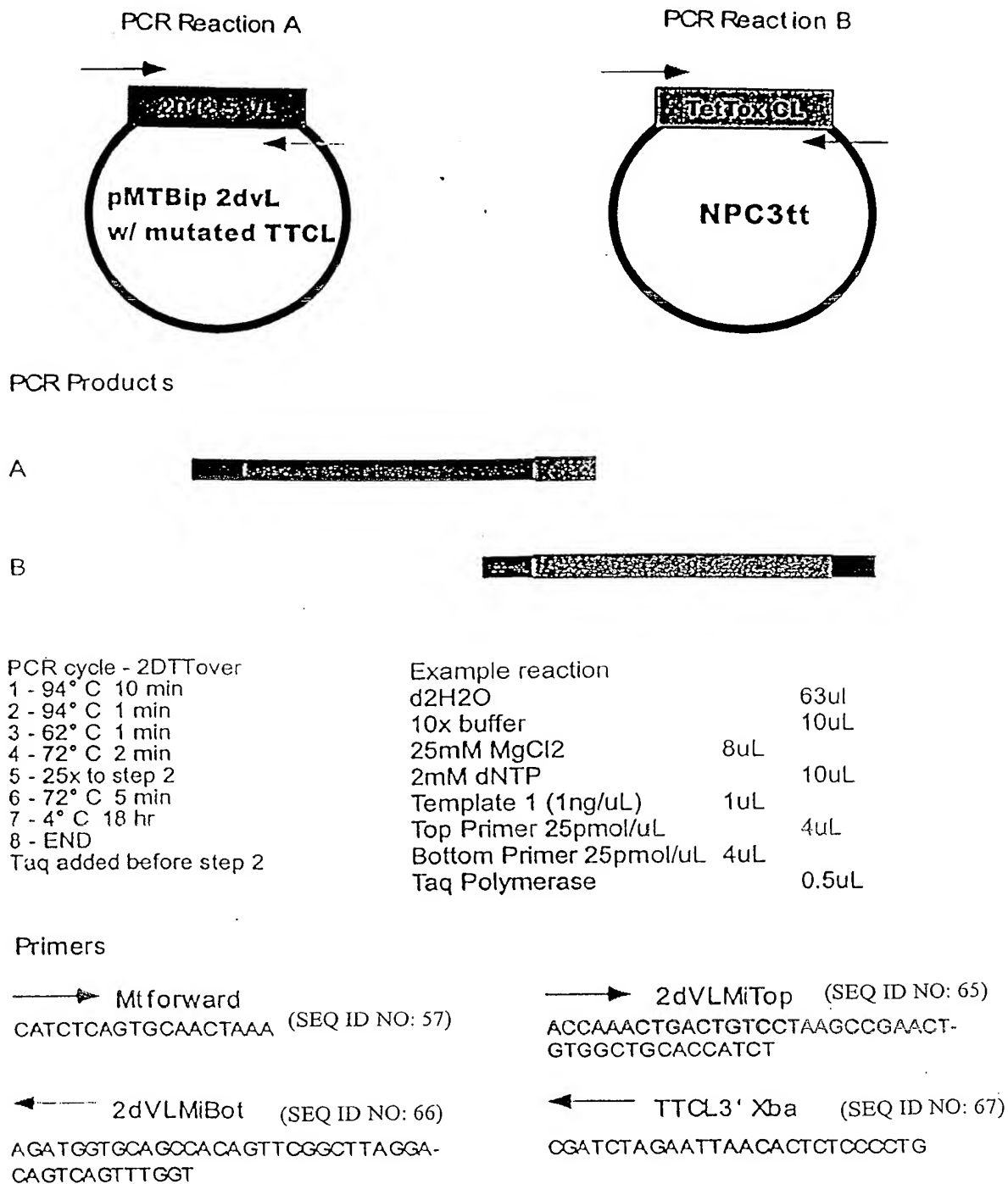
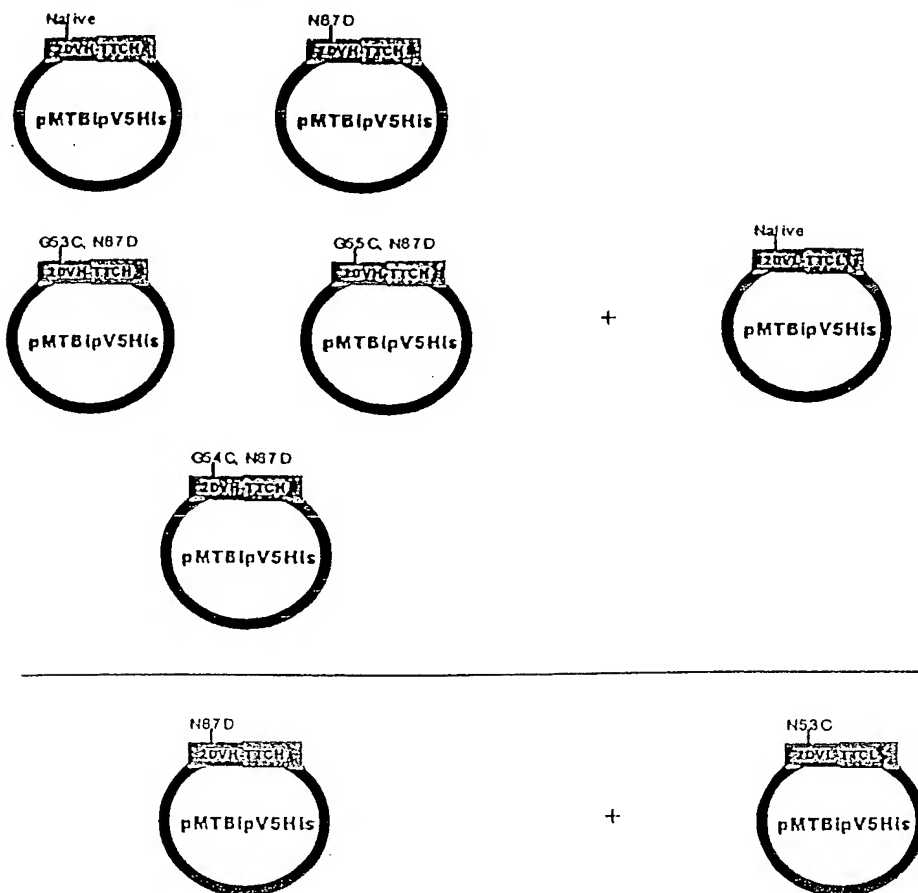


FIG. 11G

Assembled Vectors for Transfection in S2 Cells

Each of the following has been cotransfected with the native light chain:



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FIG. 12

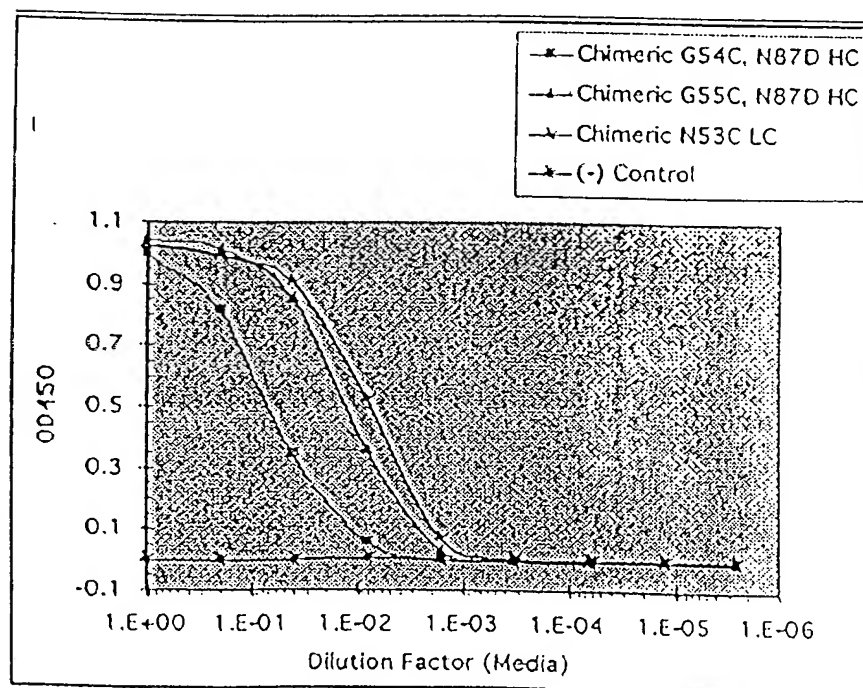
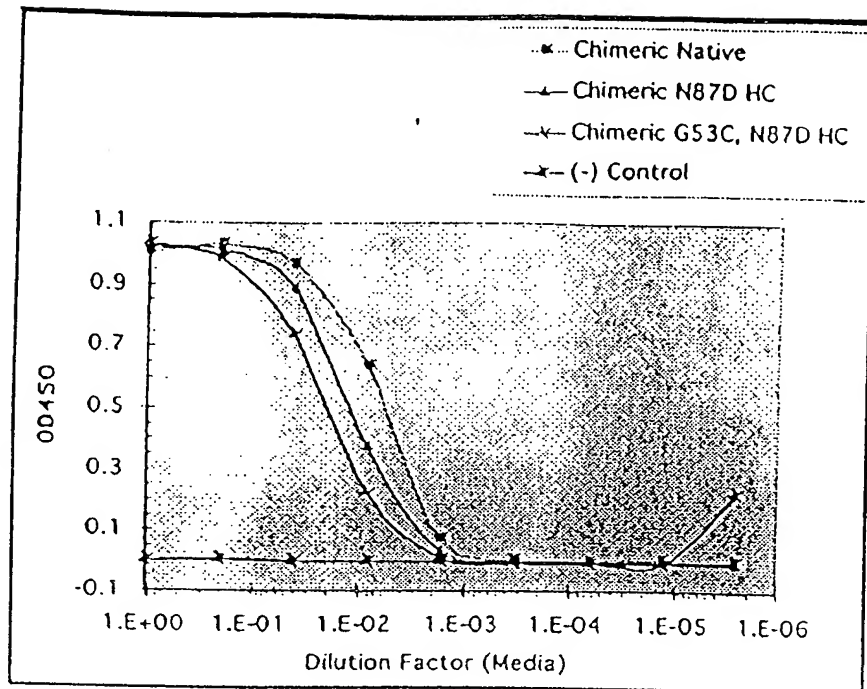


FIG. 13

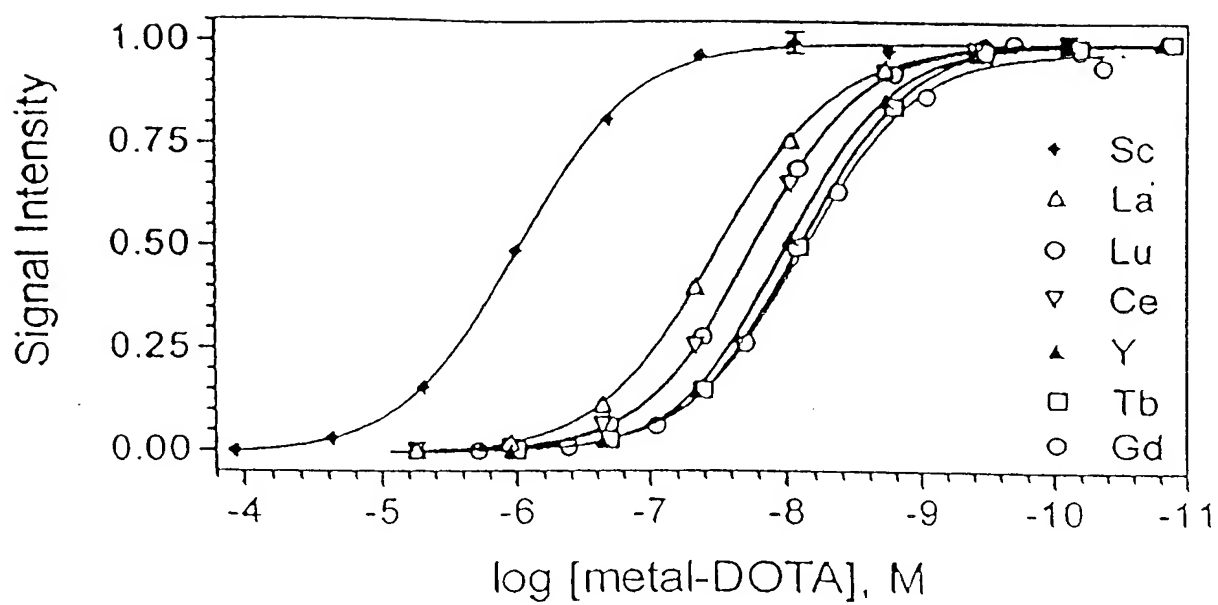


FIG. 14

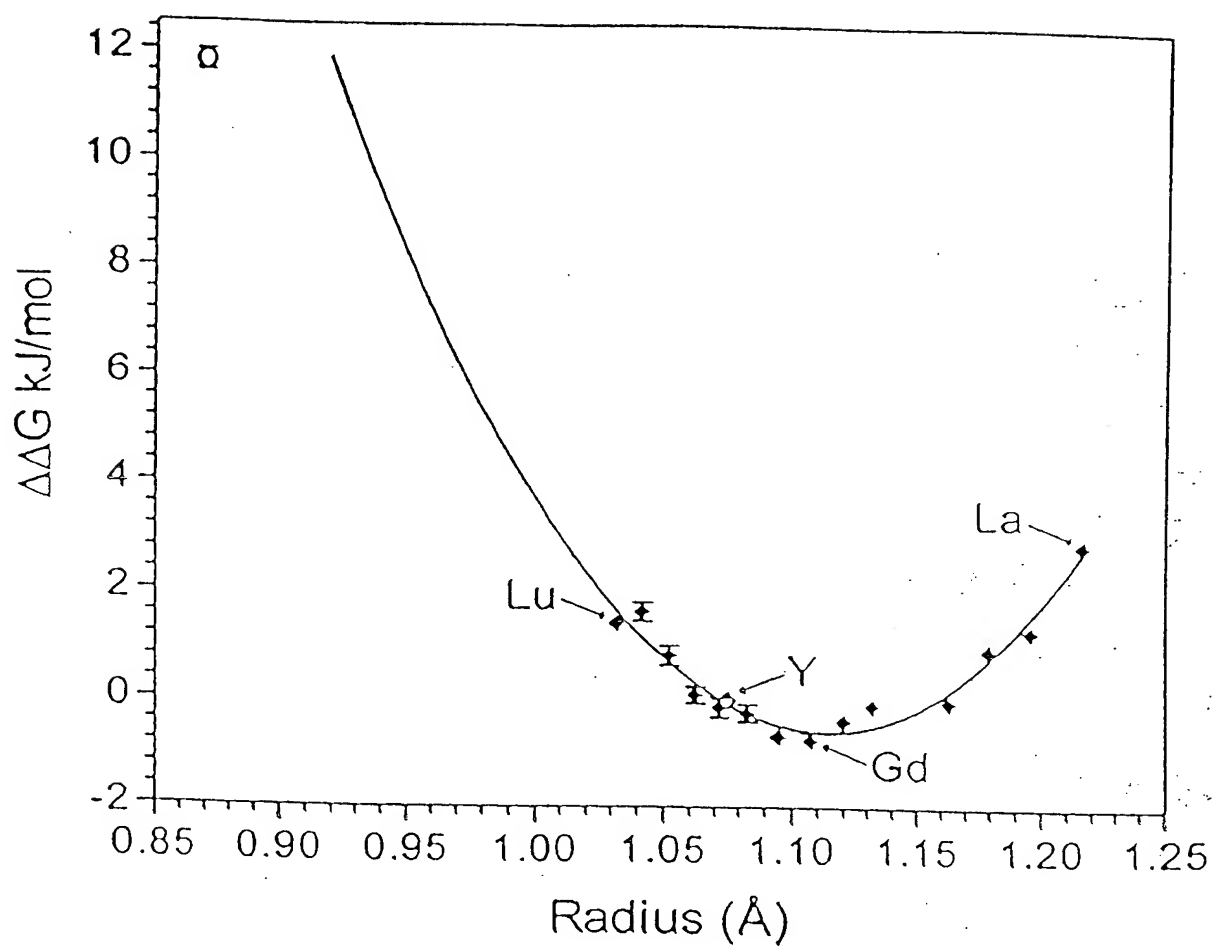


FIG. 15

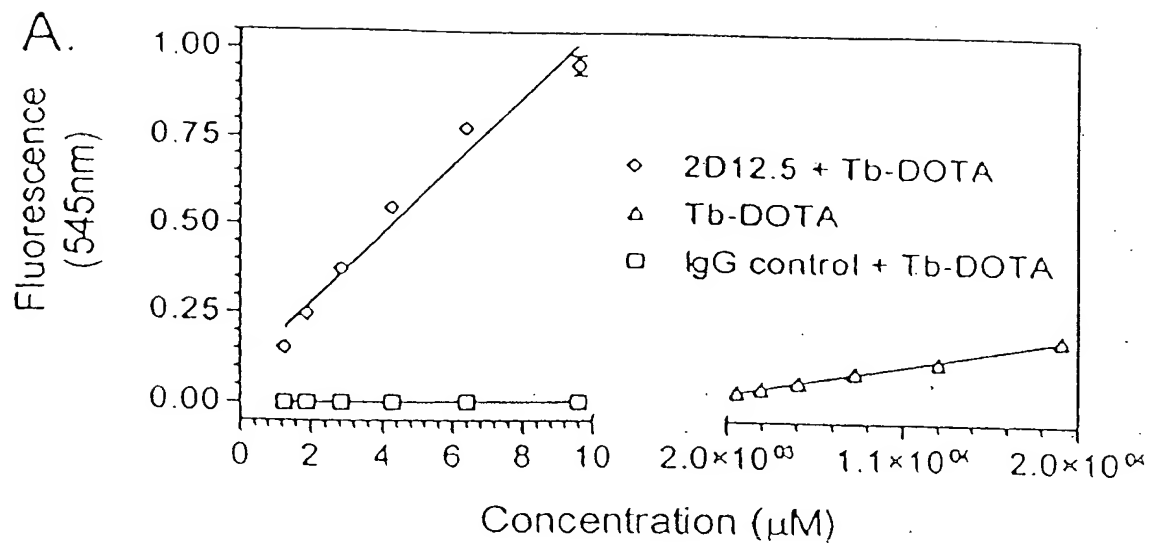
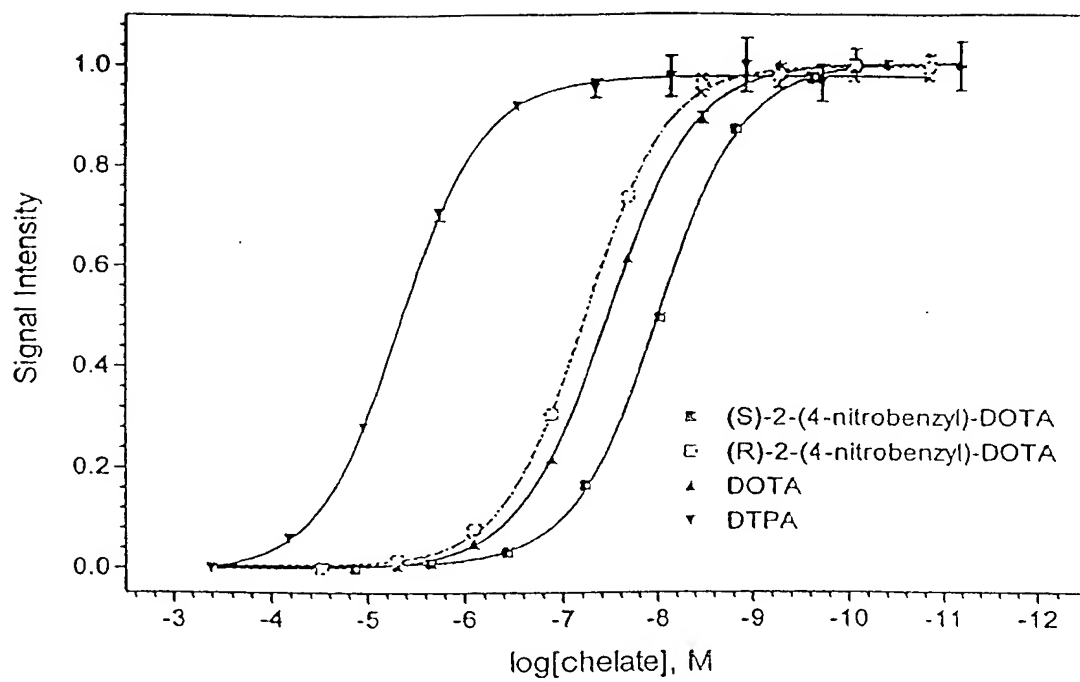


FIG. 16



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FIG. 17

